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Research Report

Development of real-time bioradiographic system for functional and metabolic imaging in living brain tissue

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ABSTRACT

We have developed a novel imaging system "real-time bioradiography", which is able to estimate the dynamic changes of physiological function and metabolism in living tissues using positron emitter-labeled tracers and chemiluminescence probes. The apparatus is comprised of a photon-counting camera, image-controller, culturing chamber, reflexible solid scintillator and temperature-controlled imaging chamber. The image distribution of radioactivity and chemiluminescence was acquirable with the reflexible solid scintillator and without, respectively. The reflexible solid scintillator is effective to exclude the affect of intra-objective different light reflectivity on radiation detection and to improve the efficiency of radiation detection. To test and to demonstrate the efficacy of this system, we examined the glucose metabolism and superoxide formation during hypoxia-reoxygenation in living brain tissues using 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) and Lucigenin, respectively. FDG uptake and chemiluminescence images were obtained at time frames of every 15 min. Glucose metabolism was enhanced during the hypoxic treatment, but the superoxide formation was enhanced during reoxygenation. The enhanced glucose metabolism during hypoxia might cause the increase in superoxide formation during reoxygenation. Thus, this new method would open up possibilities to approach simultaneous biological monitoring of a variety of biochemical events with various combinations of positron emitter-labeled tracers and chemiluminescence probes in living tissues.

1. Introduction

An autoradiographic method named "bioradiography" had been developed to estimate metabolism and physiological function in living tissues using positron emitter-labeled compounds for positron emission tomography (PET) (Matsumura et al., 1995; Murata et al., 1996; Sasaki et al., 2002a,b). This imaging technique has several advantages: (1) dynamic changes in radioactivity can be followed in living tissue, (2) physiological conditions in tissue slices can be easily controlled and changed, (3) radioligand distribution into tissue regions is not influenced by blood flow. However, momentary image acquisition is not possible using this method, because the acquisition of bioradiography is carried out by repeated exposure to radioluminographic plates. Besides, repeated exposure is troublesome.

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We here developed a novel imaging system, "real-time bioradiography", to acquire bioradiographic images of living tissue in real time using positron emitter-labeled compounds without any specific plate-changing apparatus. Moreover, the study of real-time bioradiographic imaging using radiolabeled compounds is expanded by combining it with chemiluminescence measurements using chemiluminescence probes. As an application of this system, the relation between glucose metabolism and superoxide formation during hypoxia-reoxygenation was examined in living brain tissue using FDG and Lucigenin, respectively.

2. Results

2.1. System components

Scheme of the real-time bioradiography system is drawn in Fig. 1. The arrangement of the culturing chamber and solid scintillator is illustrated in Fig. 2. The reflexible solid scintillator (Fig. 2, left) was expected to enhance the light incidence upon the photon counting camera, because light emission toward the opposite side of the camera could be additional to light emission toward the camera (Application to the Japanese Patent Agency, No. 2002-382447). Experiments have been performed to test this hypothesis in Fig. 3. Autoradiographic images of ^{18}F-labeled agar slices obtained using the intact plastic scintillator showed a different efficiency on radiation detection. The density of autoradiographic images recorded using the non-reflexible plastic scintillator was greater in non-colored agar slices than in black agar. In contrast to the intact scintillator, the density in the reflexible scintillator was not different between non-colored and black agar, and was greater than in the intact scintillator (1.2 times for non-colored and 2.2 times for black-colored agar slices) (Fig. 3, left).

The effectiveness of the reflexible scintillator was confirmed in the practical autoradiographic images obtained using FDG in brain slices (Fig. 3, right). The FDG autoradiographic image acquired with the non-reflexible scintillator showed a false distribution pattern with no significant regional differences. That image was improved by the reflexible scintillator and showed the true distribution: extensive accumulation in the cerebral cortex and little accumulation in the white matter. The detection limit for 10 min measurement and the spatial resolution for ^{18}F measurement by reflexible CaF_2(Eu) (0.5 mm-thick) under the field-of-view (3 cm × 4 cm) were 0.001 (Bq/mm^2) and 624 (μm), respectively. Other basic performances of the imaging system tested will be reported elsewhere.

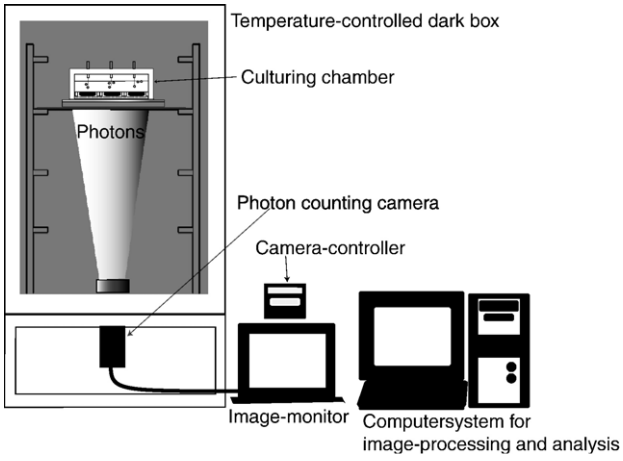

Fig. 1 – Schematic drawing of the real-time bioradiography system. Imaging of either radiation or chemiluminescence is possible with or without a reflexible solid scintillator, respectively.

2.2. Dynamic acquisition of FDG autoradiographic images of brain slices by real-time bioradiography

FDG uptake images were obtained every 15 min time up until 240 min after the start of the incubation (Fig. 4). The density of autoradiographic images in both reflexible CaF_2 (Eu) scintillator and reflexible plastic scintillator increased with the period of incubation. The efficiency of detection was higher in the reflexible CaF_2(Eu) scintillator than reflexible plastic scintillator (Figs. 4 and 5). FDG uptake (counts/15 min) in the reflexible CaF_2(Eu) scintillator was 2.7 times that in the reflexible plastic scintillator (the mean of ratios at all time points).

2.3. Combination of radiation and chemiluminescence image acquisition

Schematic diagrams of radiation and chemiluminescence combination detection are shown in Fig. 6. In the chamber containing the radioisotope and chemiluminescent probe, both radiation and chemiluminescence were emitted from the object (tissue slice). In the case of image acquisition with the reflexible solid scintillator, radiation from the object penetrates aluminum-coated Mylar foil (reflector layer) and the energy is transformed into luminescence, while chemiluminescence cannot penetrate due to the mirror effect (Fig. 6, left). Another case of image acquisition is without the reflexible solid scintillator. In this acquisition, chemiluminescence emission reaches directly into the photon counting camera, while the energy of radiation is not transformed to luminescence without scintillator (Fig. 6, right).

2.4. Glucose metabolism and superoxide formation during hypoxia-reoxygenation treatment in living brain tissue using FDG and Lucigenin

For chemiluminescence imaging, the optimum Lucigenin concentration was examined in living brain tissues during hypoxia-reoxygenation treatment. The chemiluminescence emission was enhanced dependent on the Lucigenin concentration (0.01, 0.1, 0.5, 1 and 2 mM). We decided to use 2 mM Lucigenin, since it was the maximum concentration dissolved in the medium.

As an application of the combination imaging of radiation and chemiluminescence, glucose metabolism and superoxide formation were examined during hypoxia-reoxygenation treatment in living brain slices using FDG and Lucigenin (Figs. 7 and 8). In the present study, images of radiation and chemiluminescence were acquired every 15 min separately in chambers containing different series of brain slices. A typical